Breeding Science 62: 38–45 (2012)
doi:10.1270/jsbbs.62.38

Development of diversity array technology (DArT) markers for assessment of population structure and diversity in *Aegilops tauschii*

Quahir Sohail[1)], Tariq Shehzad[2)], Andrezj Kilian[3)], Amin Elsadig Eltayeb[1)], Hiroyuki Tanaka[4)] and Hisashi Tsujimoto*[1)]

[1)] *Laboratory of Molecular Breeding, Arid Land Research Center, Tottori University*, 1390 Hamasaka, Tottori 680-0001, Japan
[2)] *Laboratory of Plant Genetics and Breeding Science, Graduate School of Life and Environmental Sciences, University of Tsukuba*, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan
[3)] *Diversity Array Technology Pty. Ltd.*, P.O. Box 7141 Yarralumla, ACT 2006, Australia
[4)] *Laboratory of Plant Genetics, Faculty of Agriculture, Tottori University*, 4-101 Minami, Koyama, Tottori 680-8553, Japan

Aegilops tauschii Coss. is the D-genome donor to hexaploid bread wheat (*Triticum aestivum*) and is the most promising wild species as a genetic resource for wheat breeding. To study the population structure and diversity of 81 *Ae. tauschii* accessions collected from various regions of its geographical distribution, the genomic representation of these lines were used to develop a diversity array technology (DArT) marker array. This *Ae. tauschii* array and a previously developed DArT wheat array were used to scan the genomes of the 81 accessions. Out of 7500 markers (5500 wheat and 2000 *Ae. tauschii*), 4449 were polymorphic (3776 wheat and 673 *Ae. tauschii*). Phylogenetic and population structure studies revealed that the accessions could be divided into three groups. The two *Ae. tauschii* subspecies could also be separately clustered, suggesting that the current taxonomy might be valid. DArT markers are effective to detect very small polymorphisms. The information obtained about *Ae. tauschii* in the current study could be useful for wheat breeding. In addition, the new DArT array from this *Ae. tauschii* population is expected to be an effective tool for hexaploid wheat studies.

Key Words: *Aegilops tauschii*, *Triticum aestivum*, synthetic wheat, DArT markers, drought tolerance.

Introduction

Aegilops tauschii Coss. is a wild, diploid ($2n = 2x = 14$, DD), self-pollinated species that is considered to be the D-genome donor to hexaploid wheat, also known as bread wheat or common wheat (*Triticum aestivum* L.; $2n = 6x = 42$, AABBDD) (Kihara 1944). *Aegilops tauschii* is adapted to a variety of environments such as desert margins, steppe regions, stony hills, wastelands, roadsides, sandy shores and even humid temperate forests (van Slageren 1994). It is also found in the edges of wheat fields in eastern Turkey, Iraq, Iran, Pakistan, India (Kashmir), China (the Himalaya), Afghanistan, most of central Asia, Transcaucasia and the Caucasus region (Feldman 2001). Because it carries one of the genomes of bread wheat, *Ae. tauschii* is regarded as the most suitable species for wheat improvement among the wild species in the tribe Triticeae. The diversity of the D genome of this species is much larger than that of bread wheat and includes many useful genes for resistance to biotic and abiotic stresses and for seed storage proteins (Assefa and Fehrmann 2000, Colmer *et al*. 2006, Cox *et al*. 1994, Lubbers *et al.*1991, Naghavi and Mardi 2010, Pestsova *et al.* 2000, Reif *et al.* 2005, Sohail *et al.* 2011). Thus, *Ae. tauschii* needs to be studied in detail in order to use it most effectively as a germplasm source for the improvement of bread wheat.

Based on morphology, taxonomists have divided *Ae. tauschii* into two subspecies: ssp. *tauschii* and ssp. *strangulata* (Eig) Tzvel. (Eig 1929, Hammer 1980). Ssp. *strangulata* has quadrate spikelets whereas ssp. *tauschii* has elongated spikelets (Kimber and Feldman 1987, Matsuoka *et al.* 2009). Ssp. *tauschii* is further divided into three morphological varieties: *anathera*, *meyeri* and *typica*, whereas ssp. *strangulata* is monotypic. Dvorak *et al.* (1998) pointed out that some *Ae. tauschii* accessions have intermediate forms and suggested that these might have a hybrid origin. The geographical distribution is also different between the two subspecies. Most ssp. *strangulata* is limited to the Caucasus and southeastern Caspian costal region, while ssp. *tauschii* is distributed throughout central and western Asia (Dudnikov and Kawahara 2006, Eig 1929). Ssp. *strangulata* is considered to be more closely related to bread wheat compared to ssp. *tauschii* (Dvorak *et al.*1998, Nishikawa *et al.* 1980, Pestsova *et al.* 2000).

Communicated by N. Mori
Received September 26, 2011. Accepted December 3, 2011.
*Corresponding author (e-mail: tsujim@alrc.tottori-u.ac.jp)

The diversity of *Ae. tauschii* has been studied using molecular tools such as chloroplast DNA variation (Matsuoka *et al.* 2008, 2009, Takumi *et al.* 2009), AFLP (Mizuno *et al.* 2010), SSR (Naghavi and Mardi 2010), isozymes (Dudnikov and Kawahara 2006) and random amplified polymorphic DNA (RAPD) markers (Okuno *et al.* 1998). Here, we used diversity array technology (DArT) markers to study the population structure and diversity of *Ae. tauschii*. DArT is a sequence-independent system, based on microarray hybridization that can be used to carry out a whole-genome scan. The method is based on the use of "genomic representations", which are DNA samples produced by using a specific combination of restriction enzymes and PCR primers. The output result is (0, 1); that is, it indicates the presence or absence of each DNA fragment contained in a genomic representation within the genome of the material being tested. DArT markers are biallelic dominant markers that provide a cost-effective, time-saving method of genome-wide genotyping (Jaccoud *et al.* 2001, Kilian *et al.* 2005). These markers have been successfully used for genotyping, diversity studies, and genetic mapping in many crop species (Jing *et al.* 2009).

The molecular information provided by the present DArT analyses will be useful for breeding programs.

Materials and Methods

Plant materials and DNA isolation

Eighty-one accessions of *Ae. tauschii* collected from various regions of its geographical distribution were used in this study. Thirteen of the accessions were ssp. *strangulata*, 62 were ssp. *tauschii*, and six were of unknown subspecies (Table 1). The AE accessions were collected by the Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Germany; the AT accessions by the Faculty of Agriculture, Okayama University, Japan; the CGN accessions by the Instituut voor Plantenveredeling, Landbouwhogeschool, Wageningen, the Netherlands; the IG accessions by the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria; the KU accessions by the Germ-Plasm Institute, Faculty of Agriculture, Kyoto University, Japan; and the PI accessions by the United States Department of Agriculture (USDA). Fifty-five of these *Ae. tauschii* accessions have been used for producing synthetic wheat. These *Ae. tauschii* were kindly provided by Prof. Y. Matsuoka, Fukui Prefectural University, Japan. The accession 28H51 has been used by ICARDA for producing synthetic wheat derivatives. Accession Aus18913 has been used for mapping of chromosome arm 1DS of *Ae. tauschii* (Spielmeyer *et al.* 2000) and to construct an *Ae. tauschii* BAC library (Moullet *et al.* 1999). Total genomic DNA was extracted from 2- to 3-week-old leaves of the 81 accessions by using the CTAB method (Murray and Thompson 1980).

Preparation of DArT markers and genotyping

A total of 81 genotypes DNA were sent to Diversity Arrays Technology Pty. Ltd., Yarralumla, Australia, for array development and genotyping as described by Wenzl *et al.* (2004) and Akbari *et al*. (2006). The *Ae.tauschii* DArT markers are referred to as (aePt-). Each of the original 81 accessions was genotyped with two arrays: Wheat DArT Array Version 3.0 and the *Ae. tauschii* array developed in this study. The genomic DNA of each accession was labeled and then hybridized to the DArT arrays. The presence or absence

Table 1. *Ae. tauschii* accessions used in this study

Country (number of accessions)	Accession[a]
Afghanistan ($n = 6$)	KU2039, PI476874, KU2063, KU2035, KU2022, KU2636^{A}
Armenia ($n = 7$)	IG127015, KU2816, KU2810, KU2824, CGN10734, KU2809 ^{A}, IG126991
Azerbaijan ($n = 2$)	IG47203, KU2806
China ($n = 6$)	AT55, AT76, AT80, AT47, PI499262, PI508262
Dagestan ($n = 2$)	KU201, IG120866
Georgia ($n = 5$)	AE454, KU2826, KU2828, KU2829A, IG48042
India (Jammu and Kashmir) ($n = 1$)	IG48042
Iran ($n = 37$)	KU20-10^{M}, KU2069, KU2074^{S}, KU2075^{S}, KU2076^{S}, KU2078^{S}, KU2079^{S}, KU20-8, KU2080^{S}, KU2088^{S}, KU20-9^{S}, KU2090^{S}, KU2091^{S}, KU2092^{S}, KU2093^{S}, KU2096, KU2097, KU2098, KU2100^{M}, KU2103, KU2104, KU2105, KU2106, KU2109^{M}, KU2111, KU2124, KU2126, KU2144, KU2155, KU2156, KU2158^{M}, KU2159, KU2083, KU2101, KU2102, KU2108^{M}, IG49095
Kazakhstan ($n = 1$)	AE1090
Kyrgyzstan ($n = 1$)	IG131606
Pakistan ($n = 4$)	IG46663, CGN10768, CGN10770, CGN10767
Syria ($n = 2$)	IG46623, 28H51
Tajikistan ($n = 1$)	IG48554
Turkey ($n = 3$)	KU2132, KU2136, PI486277
Turkmenistan ($n = 2$)	IG126387, IG126489
Unknown ($n = 1$)	Aus18913^{M}

[a] Accessions without superscripts are ssp. *strangulata*; accessions with superscripts are ssp. *tauschii*. ^{A}, ssp. *tauschii* var. *anathera*; ^{M}, ssp. *tauschii* var. *meyeri*; ^{S}, ssp. *tauschii* var. *strangulata*.

of each marker was determined on the basis of the signals from the labeling and image analysis. The DArT marker data were provided to in term of 1, 0 (present, absent) fashion, as described by Akbari *et al.* (2006).

Analysis of DArT data

To determine the population structure of *Ae. tauschii*, we applied the Bayesian method by using the model-based program Structure version 2.3.3 (Falush *et al.* 2003, Pritchard *et al.* 2000) with (0, 1) data matrices. We used a burn-in length (number of cycle runs by the simulation before collecting data) of 10^4 cycles to minimize the effect of starting configuration, and a simulation run length (after the burn-in) of 10^6 cycles, and applied the admixture model option in the Structure program. We chose cluster values (K) ranging from 2 to 9; four independent runs for each value gave consistent results.

Genetic similarities between accessions were measured by DICE similarity coefficient based on the proportion of shared alleles. The phylogenetic tree was constructed by clustering accessions based on similarity matrix using the unweighted pair group method (UPGMA) with arithmetic average algorithm in the SAHN module. Bootstrap analysis was performed using 1,000 permutations in Winboot (Yap and Nelson 1996). Bootstrap values over 50 are considered significant and indicated on the phylogenetic tree.

To calculate the genetic similarities and genetic distances between the pairs of accessions, the (0, 1) data matrixes obtained for 4449 polymorphic DArT markers (details presented in the Results section) and 81 accessions were analyzed with the following formulae:

$$\text{Similarity } (S_{ij}) = (2 \times N_{ij})/(N_i + N_j)$$

$$\begin{aligned}\text{Distance} &= 1\text{–similarity} \\ &= 1 - [(2 \times N_{ij})/(N_i + N_j)] \\ &= [N_i + N_j - 2N_{ij}]/(N_i + N_j)\end{aligned}$$

Where, S_{ij} represents the similarity between the *i*th and *j*th accessions, N_{ij} represents the number of common bands present in both the N_i and N_j accessions (*i.e.*, the number of markers where 1's are present for both the N_i and N_j accessions), N_i is number of bands in the *i*th accession (number of markers with 1's in accession N_i), and N_j is number of bands in the *j*th accession (number of markers with 1's in accession N_j).

By using the program Excel (Microsoft Corporation), we calculated genetic similarity for all 3240 possible pairs [81 × (81–1)/2] of the 81 accessions using the (0, 1) data matrix consisting of 4449 rows (4449 DArT markers) and 81 columns (81 accessions). An 81 × 81 genetic similarities matrix was created in which the values of 1 on the main diagonal (representing the similarity of each accession to itself) were not considered. The values in the genetic similarities matrix were used to calculate average similarity within ssp. *strangulata* and within the three varieties of ssp. *tauschii*. The average similarities between ssp. *strangulata* and each of the varieties of ssp. *tauschii* were also calculated.

Table 2. Quality parameters of the two different types of DArT markers used to analyze 81 *Ae. tauschii* accessions

Parameter	Marker type		
	Wheat Array 3.0	*Ae. tauschii*	Overall
Total number of DArT markers	5500	2000	7500
Number of polymorphic markers	3776	673	4449
Polymorphism (%)[a]	68.6	33.7	59.3
Polymorphism information content[b]	0.246	0.329	0.259
P (%)[c]	81.2	82.9	81.5
Reproducibility (%)[d]	99.9	99.8	99.9
Call rate (%)[e]	97.8	97.6	97.8

[a] Number of polymorphic markers/total number of DArT markers tested.
[b] Measure of polymorphism to describe the usefulness of a marker.
[c] Reflects how well the two phases of the marker are separated.
[d] On the basis of scoring for replicated samples.
[e] The number of genotypes present and not missing for certain marker.

Results

Polymorphism of DArT markers in Ae. tauschii accessions

A total of 7500 DArT markers were tested. Of these, 5500 markers from wheat DArT Array Version 3.0 (wheat markers) were developed from hexaploid bread wheat and the other 2000 (*Ae. tauschii* markers) were developed from the 81 *Ae. tauschii* accessions by the DArT company (Table 2). Of these markers, 3776 of the wheat markers (68.6%) and 673 of the *Ae. tauschii* markers (33.7%) showed polymorphism among the 81 accessions of *Ae. tauschii*. The polymorphism information content (PIC) of the DArT markers ranged from 0.024 to 0.500 per marker, with an average of 0.259 (Table 2). Thirty-two markers had a very low PIC (0.024), which means that these markers showed little polymorphism. About 96% of the markers had a call rate of more than 90% and 48% of the markers had a call rate of 100%; 95% of the wheat markers had a call rate of 90% or more while almost all of the *Ae. tauschii* markers had a call rate of more than 87%. To verify the reproducibility of the genotyping, two of the accessions were analyzed in duplicate (*i.e.*, two wells per accession). The results of both pairs were identical, except for a few missing data points.

Phylogenetic tree based on DArT marker genotypes

Phylogenetic trees were constructed using the 3776 polymorphic wheat markers, the 673 polymorphic *Ae. tauschii* markers and the combined total of 4449 markers showing polymorphism. The structures of these three trees were very similar, therefore, the tree made by using all 4449 markers is shown in Fig. 1.

The *Ae. tauschii* population could be clearly divided into three groups designated A, B and C. Group A contained accessions from China, central Asia (Kazakhstan, Kirghizstan, Tajikistan and Turkmenistan), Afghanistan, Pakistan, India and western Asia (Georgia, Armenia, Syria) and only three

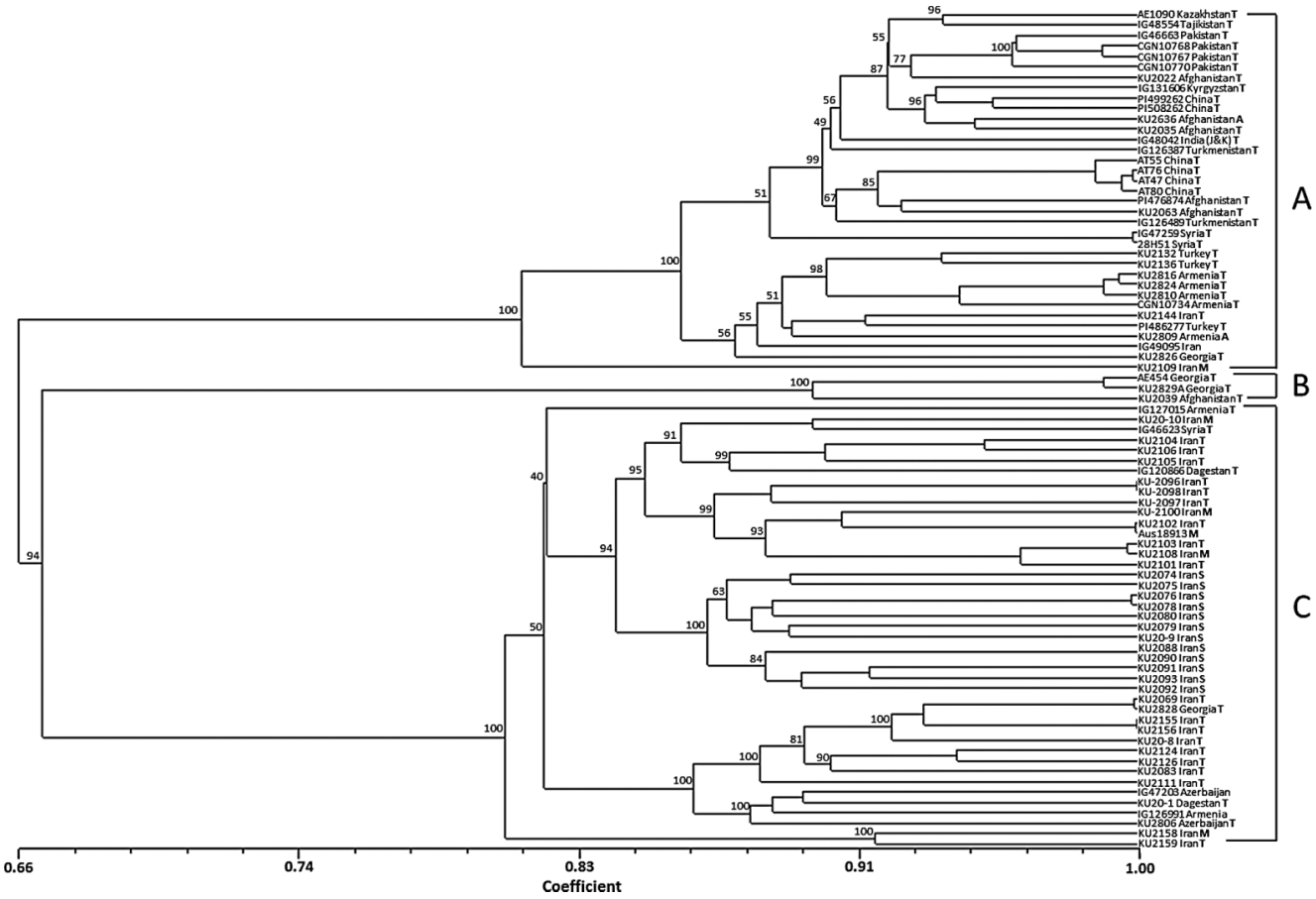

Fig. 1. Phylogenetic tree of the 81 *Ae. tauschii* accessions constructed using 4449 DArT markers.

accessions (KU2144, IG49095 and KU2109) were from Iran. Group B had three accessions, two from Georgia and one from Afghanistan. Accessions in group C were from Iran, with the exception of accession KU2828, IG46623, IG120866 and IG127015, which were from western Asia (Georgia, Syria, Dagestan and Armenia, respectively).

Sixty-six of the accessions in this study belonged to ssp. *tauschii*, of which 58 were var. *typica*, 6 var. *meyeri* and 2 var. *anathera* (Fig. 1). Twelve accessions were classified as ssp. *strangulata* and the remaining 3 accessions were of unknown variety. All of the accessions of ssp. *strangulata* clustered in one clade in Group C. On the other hand, the three varieties of ssp. *tauschii* did not cluster into a particular clade. Accessions classified as var. *anathera* were present only in Group A, while those classified as var. *meyeri* appeared in Group C, with only one (KU2109) in Group A. Using this tree, we could deduce that the 3 accessions without species information belong to ssp. *tauschii*. Accession IG127015 from Armenia was classified into Group C but was separate from the other accessions in this group. Accessions KU2039 (Afghanistan), AE454 (Georgia) and KU2829A (Georgia) were classified into Group B (Fig. 1).

Structural analysis of the Ae. tauschii population

The structure of the *Ae. tauschii* population was further studied to assess the degree of relatedness among the accessions and to group genetically similar accessions (Fig. 2). For this purpose, we used the model-based program Structure version 2.3.3 (Falush *et al.* 2003, Pritchard *et al.* 2000). Four independent runs yielded consistent results. Values of K (number of clusters) ranging from 2 to 9 were tested. The values log-likelihood for the observed data from $K = 2$ to 9 are: −77679.6, −72394.1, −538662.9, −1840057.9, −111610.2, −4742273, −958412 and −1890751, respectively. $K = 3$ was selected as having the highest log-likelihood for the observed data, indicating that the current *Ae. tauschii* population can be clearly divided into three groups (Fig. 2). One of the three groups contained the accessions KU2829A, AE454 and KU2039, which are quite distant from the others. Interestingly, these lines carry useful traits for drought tolerance (Sohail *et al*. 2011, discussed later in detail). The third group corresponds to Group C in the phylogenetic tree, which includes all of the accessions of ssp. *strangulata* and about half (29) of the accessions of ssp. *tauschii*.

Genetic relatedness and dissimilarity among subspecies and varieties

We also analyzed the relatedness and dissimilarity between the two subspecies of *Ae. tauschii* and among the three varieties in ssp. *tauschii*. The average similarity among

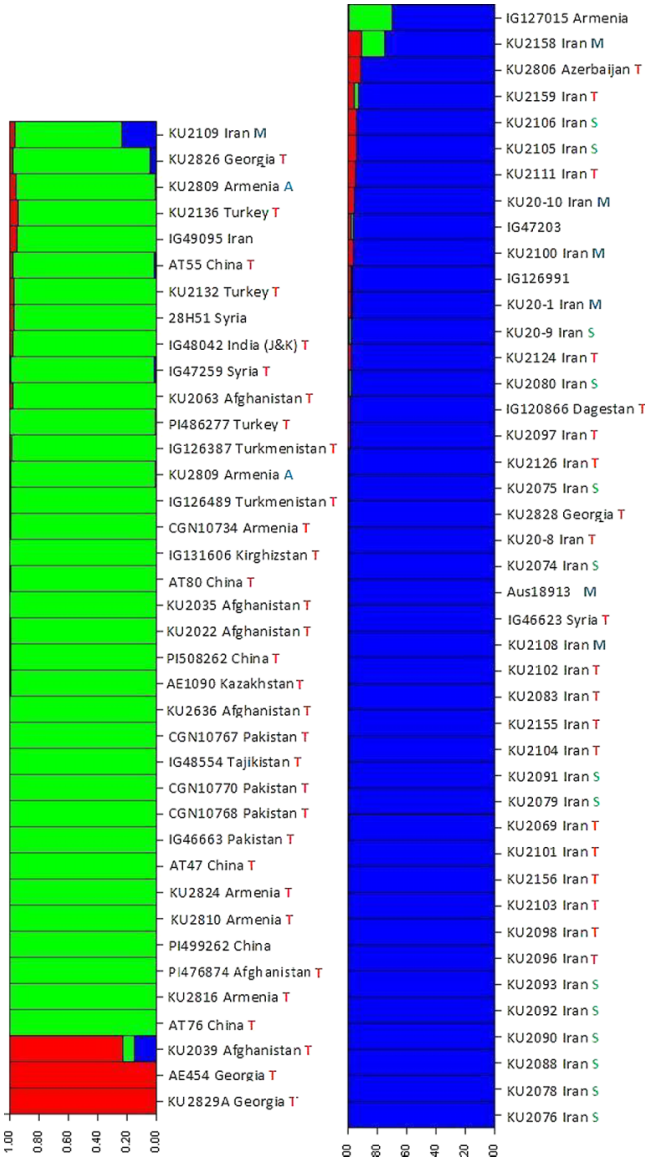

Fig. 2. Analysis of the population structure of 81 *Ae. tauschii* accessions constructed using 4449 polymorphic DArT markers and with the number of clusters (*K*) set to 3.

those in ssp. *strangulata* was 0.87, while that in ssp. *tauschii* was 0.74. Though the average similarity between accessions of var. *typica* and accessions of ssp. *strangulata* was low, the range was wide (0.61 to 0.99), indicating that some of ssp. *tauschii* var. *typica* had great similarity with some accessions of ssp. *strangulata*. Another interesting observation that var. *typica* had very high maximum similarity (0.99) with var. *meyeri* and ssp. *strangulata*. Some of the *Ae. tauschii* accessions had very high genetic similarities (0.99): AT47, AT76 and AT80; KU2096 and KU2098; KU2156 and KU2155; KU2088 and KU2090; KU2108 and KU2103; Aus18913 and KU2102; CGN10767 and CGN10768; KU2076 and KU2078; KU2816 and KU2824 and KU2069 and KU2828.

Discussion

Polymorphism of DArT markers from wheat and Ae. tauschii

The DArT method for performing whole-genome scans is proving to be useful for the studies of many plant species. It is currently being used for diversity studies, population structure analysis, mapping, marker-assisted selection for multiple phenotypic traits, *etc.* (Howard *et al.* 2011). We tested 5500 previously developed wheat markers (Wheat Array 3.0) and 2000 *Ae. tauschii* markers that were produced in this study to examine the diversity of *Ae. tauschii*. Out of the 7500 total markers tested, 4449 showed polymorphism among the 81 accessions in this study (Table 2). The wheat markers showed a higher percentage of polymorphism (68.6%) than the *Ae. tauschii* markers (33.7%). This might be because the wheat markers had previously been screened and selected as good markers for the D genome of common wheat. However, Pestsova *et al*. (2000) also had a similar result in a study using SSR markers that had not been prescreened and preselected: the SSR markers developed based on *T. aestivum* sequences revealed a higher level of polymorphism in *Ae. tauschii* accessions than the markers developed from *Ae. tauschii* itself.

Previous studies using DArT markers in cultivated crops showed less polymorphism than we found in the current study; for example, the polymorphism rate in hop (*Humulus lupulus* L.) was 11.9% (Howard *et al.* 2011); sugarcane (*Saccharum officinarum*), 7.0% (Heller-Uszynska *et al.* 2010); wheat (*T. aestivum*), 9.4% (Akbari *et al.* 2006); cassava (*Manihot esculenta*), 14.6% (Xia *et al.* 2005) and barley (*Hordeum vulgare*), 10.4% (Wenzl *et al.* 2004). Badea *et al.* (2011) reported the polymorphism rate of triticale for DArT markers of hexaploid wheat, triticale, and rye as 8.6%, 23.4% and 23.8%, respectively. Compared to these studies, we found higher polymorphism rate for *Ae. tauschii*. This might be because *Ae. tauschii* is a wild species and because the collection used here is representative of the wide diversity of *Ae. tauschii.* DArT markers of related species can be used for crops and vice versa; for example, Jing *et al.* (2009) reported that *T. monococcum* DArT markers can be effectively used for hexaploid and tetraploid wheat as well as for diploid *Triticum* species.

Classification of Ae. tauschii accessions on the basis of country of origin

The phylogenetic tree of *Ae. tauschii* made in this study contained three largely differentiated groups, A, B and C. Most accessions in Group A were from regions other than Iran. The region encompassed by Group A starts at the Afghanistan–Turkmenistan border, extends through southern Uzbekistan, Tajikistan and Kirghizstan and reaches all the way to southern Kazakhstan. Some patches of this group are distributed along the Pakistan–Afghanistan border, in India (Jammu and Kashmir), and in western and central China. Group B only had three accessions (AE454 and KU2829A from Georgia and KU2039 from Afghanistan)

these accessions also formed an independent group in the structure analysis. Two of these lines (AE454 and KU2829A) were reported to be in an unclear genealogical position by Matsuoka *et al.* (2008, 2009) and Mizuno *et al.* (2010). Most accessions in Group C were from Iran, mainly around the lower western and southern sides of the Caspian Sea and along the Iran–Turkmenistan border. Mizuno *et al.* (2010) has also reported a similar grouping on the basis of AFLP results. It is noteworthy that the accessions in Group C were collected from areas with a Mediterranean climate whereas the accessions in Group A were collected from areas with an arid or semiarid steppe climate.

Most of the accessions originating from the same country were clustered together in the phylogenetic tree (Fig. 1). Among the six accessions from China, two (PI499262 and PI508262) were clustered together with those from Afghanistan and Kirghizstan and four (AT55, AT80, AT76 and AT47) were clustered together with those from Afghanistan and Turkmenistan. The first two were collected from Xingjian, which borders with Afghanistan and Kirghizstan, and the other four were from Shaanxi province. The accessions from Xinjiang must have been found in the natural distribution area of this species. However, the accessions from Shaanxi might be weedy types that were carried from Turkmenistan by human activity at some point in history. Likewise, some accessions showed high genetic similarity despite being collected from distant sites. For example, KU2069 from Iran and KU2828 from Georgia were very similar (0.99). This suggests the occurrence of migration, as *Ae. tauschii* has a weedy growth habit and can occur in a mixture with wheat.

The origin of Aus18913, a key accession for genome sequencing, was not known when we began our study. However, our data clearly indicated that it originated from Iran because of its close similarity (0.99) to KU2102, which was collected at 52 km northwest of Ramsar, Iran, on the southwestern coast of the Caspian Sea (http://www.shigen.nig.ac.jp/wheat/komugi/strains/nbrpDetailAction.do?strainId=KU-2102).

Classification of subspecies

Accessions classified as ssp. *strangulata* clearly clustered together in the phylogenetic tree (Fig. 1), despite the difficulty of classification between ssp. *strangulata* and ssp. *tauschii* based on phenotype (Dudnikov and Kawahara 2006, Dvorak *et al.*1998, Pestsova *et al.* 2000), the DArT markers revealed a clear cluster of ssp. *strangulata*. This indicates that both subspecies are genetically well diverged and that this taxonomy is probably valid. Diversity analysis using thousands of DArT markers is more powerful than analysis with other markers because of the high number and high degree of polymorphism detected by these markers. Two ssp. *meyeri* accessions, Aus18913 and KU2108, clustered with accessions KU2102 and KU2103 of var. *tauschii* respectively, and had a similarity of 0.99 with each other. The reason for this might be a large number of traits with intermediate morphology (Dudnikov and Kawahara 2006, Pestsova *et al.* 2000, van Slageren 1994) caused by hybridization between the two subspecies (Dvorak *et al.*1998, Hammer 1980, Hammer and Knupffer 1979).

In ssp. *tauschii*, the varieties *anathera*, *meyeri* and *typica* could not be well differentiated based on the DArT markers. Mizuno *et al*. (2010) suggested the reason for this might be that only a small number of genetic loci control the morphological traits used to discriminate between them. Though accessions of var. *anathera* were included only in Group A, accessions of var. *meyeri* and var. *typica* appeared in both groups. On the basis of RFLP markers, Lubbers *et al.* (1991) reported that ssp. *strangulata* was more similar to var. *meyeri* than to var. *typica*. Here, no accessions of var. *meyeri* were closely grouped in the phylogenetic tree (Fig. 1).

Use of Ae. tauschii diversity information for wheat breeding

Because *Ae. tauschii* is the D-genome donor to hexaploid bread wheat, it is regarded as the most promising wild species as a genetic resource for wheat breeding (Feldman 2001, Helbaek 1959, Kihara 1944, Mujeeb-Kazi *et al*. 1996). To introduce useful genes from this wild species into common wheat, *Ae. tauschii* (DD) is crossed with durum wheat (AABB) to produce a hexaploid amphiploid (AABBDD) that is called synthetic wheat (has the same genomes as bread wheat). When using this process, we should utilize the large diversity of *Ae. tauschii* as efficiently as possible. Sohail *et al*. (2011) measured the morphological and physiological traits related to drought tolerance in many *Ae. tauschii* accessions and their synthetic hexaploid produced by crosses between *Ae. tauschii* accessions and a durum wheat, *Triticum durum* cv. Langdon. The results showed great diversity in drought response at both the diploid and the hexaploid levels. They found that synthetic wheats made by accessions from Georgia and central Asia (corresponding to Group B in this study) showed higher performance than others and that these synthetic wheats and may be useful for wheat breeding. The molecular information provided by the present DArT analyses will elucidate the genetic basis of the morphological and physiological characteristics at both ploidy levels. DArT makers have shown some lines to have very high genetic similarity (0.99); this information is very important for breeders to select suitable and more diverse material for breeding programs, to avoid duplication and save time and labor. This information is also important for gene banks to avoid preserving the same genotypes collected by different researchers and tagged with different accession numbers. For example, some of the accessions having high genetic similarity mentioned in the result section might be duplicates.

In conclusion, DArT markers are capable of detecting even very small polymorphisms, are cost-effective and are efficient for whole-genome scans and population structure. The present study will be useful for wheat breeding and will provide useful information with which to choose a range of accessions that represent a high degree of genetic diversity.

Additionally, the new array developed here, which represents a large and diverse collection of *Ae. tauschii* accessions, could be an effective tool for hexaploid wheat studies.

Acknowledgments

This work was partly supported by Science and Technology Research Partnership for Sustainable Development (SATREPS) of Japan Science and technology Agency/Japan International Cooperation Agency, and the Global Center of Excellence for Dryland Science, Tottori University.

Literature Cited

Akbari, M., P. Wenzl, V. Caig, J. Carling, L. Xia, S. Yang, G. Uszynski, V. Mohler, A. Lehmensiek, H. Kuchel *et al.* (2006) Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. Theor. Appl. Genet. 113: 1409–1420.

Assefa, S. and H. Fehrmann (2000) Resistance to wheat leaf rust in *Aegilops tauschii* Coss. and inheritance of resistance in hexaploid wheat. Genet. Resour. Crop. Evol. 47: 135–140.

Badea, A., F. Eudes, D. Salmon, S. Tuvesson, A. Vrolijk, C. T. Larsson, V. Caig, E. Huttner, A. Kilian and A. Laroche (2011) Development and assessment of DArT markers in triticale. Theor. Appl. Genet. 122: 1547–1560.

Colmer, T. D., J. Flowers and R. Munns (2006) Use of wild relatives to improve salt tolerance in wheat. J. Exp. Bot. 57: 1059–1078.

Cox, T. S., W. J. Raupp and B. S. Gill (1994) Stem rust resistance *LR*41, *LR*42, and *LR*43 transferred from *Triticum tauschii* to common wheat. Crop Sci. 34: 339–343.

Dudnikov, A. J. and T. Kawahara (2006) *Aegilops tauschii*: genetic variation in Iran. Genet. Resour. Crop. Evol. 53: 579–586.

Dvorak, J., M. C. Luo, Z. L. Yang and H. B. Zhang (1998) The structure of the *Aegilops* genepool and evolution of hexaploid wheat. Theor. Appl. Genet. 97: 657–670.

Eig, A. (1929) Monographisch-kritische Ubersicht der Gattung *Aegilops*. Repertorium Specierum Novarum Regni Vegetabilis, Beihefte. 55: 1–228.

Falush, D., M. Stephens and J. K. Pritchard (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164: 1567–1587.

Feldman, M. (2001) Origin of cultivated wheat. *In*: Bonjean, A. P. and W. J. Angus (eds.) The World Wheat Book: A History of Wheat Breeding, Lavoisier Publishing, Paris, pp. 3–53.

Hammer, K. (1980) Zur Taxonomie und Nomenklature der Gattung *Aegilops* L. Feddes Rep. 91: 225–258.

Hammer, K. and H. Knüpffer (1979) Eine Methode zur Abgrenzung infraspezifischer Sippen anhand quantitativer blütenökologischer Merkmale bei *Aegilops tauschii* Coss. Feddes Rep. 90: 179–188.

Helbaek, H. (1959) Domestication of food plants in the old world. Science 130: 365–372.

Heller-Uszynska, K., G. Uszynski, E. Huttner, M. Evers, J. Carlig, V. Caig, K. Aitken, P. Jackson, G. Piperidis, M. Cox *et al.* (2010) Diversity arrays technology effectively reveals DNA polymorphism in a large and complex genome of sugarcane. Mol. Breed. 28: 37–35.

Howard, E. L., S. P. Whittock, J. Jakse, J. Carling, P. D. Matthews, G. Probasco, J. A. Henning, P. Darby, A. Cerenak, B. Javornik *et al.* (2011) High-throughput genotyping of hop (*Humulus lupulus* L.) utilising diversity arrays technology (DArT). Theor. Appl. Genet. 122: 1265–1280.

Jaccoud, D., K. Peng, D. Felnstein and A. Killian (2001) Diversity arrays: a solid state technology for sequence information independent genotyping. Nucleic Acids Res. 29: e25.

Jing, H. C., C. Bayon, K. Kanyuka, S. Berry, P. Wenzl, E. Huttner, A. Kilian and K. E. Hammond-Kosack (2009) DArT markers: diversity analyses, genomes comparison, mapping and integration with SSR markers in *Triticum monococcum*. BMC Genomics 10: 458–474.

Kihara, H. (1944) Discovery of the DD-analyser, one of the ancestors of *Triticum vulgare*. Agric. Hort. 19: 13–14.

Kilian, A., E. Huttner, P. Wenzl, D. Jaccoud, J. Carling, V. Caig, M. Evers, K. Heller-Uszynska, C. Cayla, S. Patarapuwadol *et al.* (2005) The fast and the cheap: SNP and DArT-based whole genome profiling for crop improvement. *In*: Tuberosa, R., R. L. Phillips and M. Gale (eds.) Proceedings of the International Congress "In the wake of the double helix: from the green revolution to the gene revolution", 27–31 May 2003, Avenue Media, Bologna, Italy, pp. 443–461.

Kimber, G. and M. Feldman (1987) Wild wheat: an introduction. Special report 353 College of Agriculture University of Missouri, Columbia. pp. 66–67.

Lubbers, E. L., K. S. Gill, T. S. Cox and B. S. Gill (1991) Variation of molecular markers among geographically diverse accessions of *Triticum tauschii*. Genome 34: 354–361.

Matsuoka, Y., S. Takumi and T. Kawahara (2008) Flowering time diversification and dispersal in Central Eurasian wild wheat *Aegilops tauschii* Coss.: Genealogical and ecological framework. PLoS One 3(9): e3138.

Matsuoka, Y., E. Nishioka, T. Kawahara and S. Takumi (2009) Genealogical analysis of subspecies divergence and spikelet-shape diversification in central Eurasian wild wheat *Aegilops tauschii* Coss. Plant Syst. Evol. 279: 233–244.

Mizuno, N., M. Yamasaki, Y. Matsuoka, T. Kawahara and S. Takumi (2010) Population structure of wild wheat D-genome progenitor *Aegilops tauschii* Coss.: implications for intraspecific lineage diversification and evolution of common wheat. Mol. Ecol. 19: 999–1013.

Moullet, O., H. B. Zhango and E. S. Lagudah (1999) Construction and characterisation of a large DNA insert library from the D genome of wheat. Theor. Appl. Genet. 99: 305–313.

Mujeeb-Kazi, A., V. Rosas and S. Roldan (1996) Conservation of the genetic variation of *Triticum tauschii* in synthetic hexaploid wheats and its potential utilization for wheat improvement. Genet. Resour. Crop Evol. 43: 129–134.

Murray, M. G. and W. F. Thompson (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8: 4321–4326.

Naghavi, M. R. and M. Mardi (2010) Characterization of genetic variation among accessions of *Aegilops tauschii*. AsPac J. Mol. Biol. Biotechnol. 18: 93–96.

Nishikawa, K., Y. Furuta and T. Wada (1980) Genetic studies on alpha-amylase isozymes in wheat. III. Intraspecifc variation in *Aegilops squarrosa* and birthplace of hexaploid wheat. Jpn. J. Genet. 55: 325–336.

Okuno, K., K. Ebana, B. Noov and H. Yoshida (1998) Genetic diversity of Central Asian and North Caucasian *Aegilops* as revealed by RAPD markers. Genet. Resour. Crop. Evol. 45: 398–394.

Pestsova, E., V. Korzun, N. P. Goncharov, K. Hammer, M. W. Ganal and M. S. Roder (2000) Microsatellite analysis of *Aegilops tauschii* germplasm. Theor. Appl. Genet. 101: 100–106.

Pritchard, J. K., M. Stephens and P. Donnelly (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945–

959.

Reif, J.C., P. Zhang, S. Dreisigacker, M.L. Warburton, M. van Ginkel, D. Hoisington, M. Bohn and A.E. Melchinger (2005) Wheat genetic diversity trends during domestication and breeding. Theor. Appl. Genet. 110: 859–864.

Sohail, Q., T. Inoue, H. Tanaka, A.E. Eltayeb, Y. Matsuoka and H. Tsujimoto (2011) Applicability of *Aegilops tauschii* drought tolerance traits to breeding of hexaploid wheat. Breed. Sci. 61: 347–357.

Spielmeyer, W., O. Moullet, A. Laroche and E.S. Lagudah (2000) Highly recombinogenic regions at seed storage protein loci on chromosome 1DS of *Aegilops tauschii*, the D-genome donor of wheat. Genetics 155: 361–367.

Takumi, S., E. Nishioka, H. Morihiro, T. Kawahara and Y. Matsuoka (2009) Natural variation of morphological traits in the wild wheat progenitor *Aegilops tauschii* Coss. Breed. Sci. 59: 579–588.

van Slageren, M.W. (1994) Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. et Spach) Eig (Poaceae). ICARDA and Wageningen Agric. Univ.

Wenzl, P., J. Carling, D. Kudrna, D. Jaccoud, E. Huttner, A. Kleinhofs and A. Kilian (2004) Diversity arrays technology (DArT) for whole-genome profiling of barley. Proc. Natl. Acad. Sci. USA 101: 9915–9920.

Xia, L., K. Peng, S. Yang, P. Wenzl, M. Carmen De Vicente, M. Fregene and A. Kilian (2005) DArT for high-throughput genotyping of cassava (*Manihot esculenta*) and its wild relatives. Theor. Appl. Genet. 110: 1092–1098.

Yap, I.K. and R.J. Nelson (1996) WinBoot: a program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA-based dendrograms. IRRI. Disc. Ser. No. 14 Int. Rice Res. Inst., Manila, Philippines.